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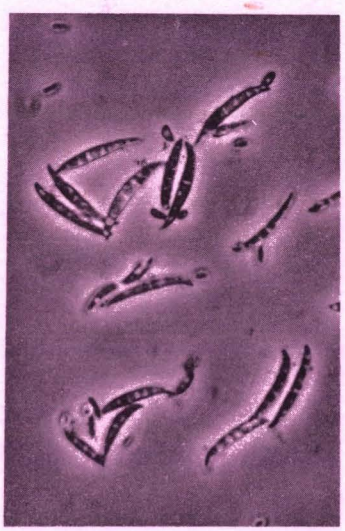
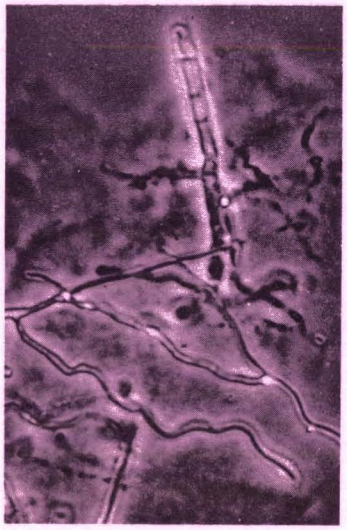
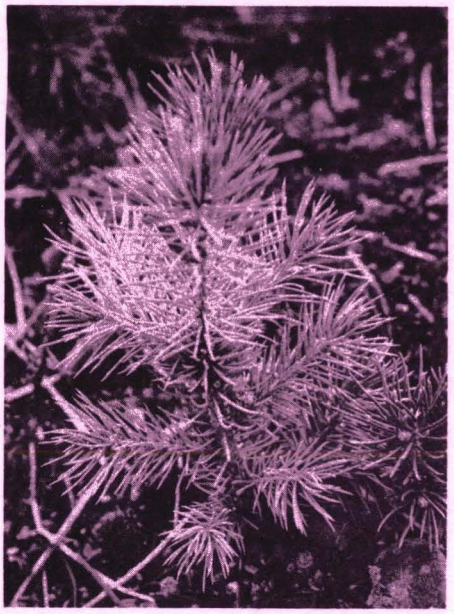
SEEDLING DISEASE AT COEUR D'ALENE FOREST NURSERY AND ITS RELATION TO OUTPLANTING SURVIVAL







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The upper left photograph shows macro- and microconidia of *Fusarium oxysporum*; the lower left shows mycelium of the fungus; the right shows the disease caused by the fungus on Engelmann spruce.

SEEDLING DISEASE AT COEUR D'ALENE FOREST NURSERY AND ITS RELATION TO OUTPLANTING SURVIVAL

by

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Environmental Services

ABSTRACT

Diseased conifer nursery stock, including Douglas-fir, western larch, and Engelmann spruce, was selected from the U.S. Forest Service nursery at Coeur d'Alene, Idaho, to determine (1) the most probable cause of disease, (2) the fate of outplanted diseased stock, and (3) the height growth of outplanted diseased seedlings. *Fusarium* spp. were isolated at high frequency--up to 90 percent--from diseased and healthy seedlings and were considered the most probable causal agent. Both shoot and and root tissue were infected. Mortality of outplanted stock after three growing seasons was greater for seedlings originally classified as diseased than for healthy. The difference was most pronounced in western larch where 50 percent of diseased seedlings died compared to 30 percent of the healthy. The diseased stock that apparently recovered after two growing seasons had about the same height growth as controls, whereas seedlings not recovered had very poor growth. It is postulated that poor soil conditions in the nursery are responsible for the disease problem.

INTRODUCTION

The U.S. Forest Service forest tree nursery at Coeur d'Alene, Idaho, has sustained a disease problem in conifer seedlings since 1964. The situation was recognized as serious when late in 1969 Steve McDonald, Coeur d'Alene nurseryman, reported that over 70 percent of the Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), western larch (*Larix occidentalis* Nutt.), and Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) in parts of the nursery were exhibiting symptoms of root disease. These included chlorosis and necrosis of foliage (primarily near the seedling top), abnormally short needle length, wilt, and mortality. Many of the lateral roots of all affected seedlings were discolored brownish or blackish, and the discoloration often extended into the tap root.

During the fall of 1969, at McDonald's request, a small sample of diseased seedlings was collected at the nursery by Region 1 plant pathologists. Isolations for possible fungal pathogens were made from diseased and healthy 2-0[1/] and 1-0 Douglas-fir, western larch, and

1/ 2-0 means 2 years in nursery bed, 0 years in transplant bed.

Engelmann spruce. *Fusarium oxysporum* Schlecht. and *F. roseum* Lk. ex Fr. emend. Snyd. and Hans. were recovered consistently from both healthy and diseased seedlings, and *Verticillium* sp. and *Botrytis* sp. were occasionally found. A small lot of each seedling species and the soil in which each was growing were sent to Dr. Jerry Riffle, nematologist at the Rocky Mountain Forest and Range Experiment Station, Albuquerque, New Mexico. He reported no plant pathogenic nematode activity, but did find in the soil samples numerous fungal sclerotia.2/ He suggested a fungal problem likely exists.

Seedlings grown at the Coeur d'Alene Nursery are shipped to many parts of Region 1. Steve McDonald explained that the most severely affected seedlings would be culled during the sorting operation prior to shipping. However, the slightly-to-moderately affected and the healthy would all be sent to the Forests for outplanting on cutover areas. The question that arose was, would infected seedlings survive outplanting? This study was designed in relation to that query. Our objectives were:

1. To identify the most probable disease causal agent.

2. To determine the fate of outplanted healthy and diseased Douglas-fir, western larch, and Engelmann spruce seedlings.

3. To determine height growth of outplanted healthy and diseased seedlings of the species mentioned above.

MATERIALS AND METHODS

Outplanting.--Three species of seedlings at three different disease intensities were selected for study. The species were 2-0 Douglas-fir, 2-0 Engelmann spruce, and because of its rapid growth in the nursery, 1-0 western larch. The disease intensities were:

1. Control - no chlorosis or necrosis of foliage, no wilting. Obtained at Montana State Nursery, Missoula, Montana.

2. Healthy - little or no chlorosis of foliage at terminal, no wilting, apparently disease-free. Obtained at Coeur d'Alene Nursery.

3. Diseased - obvious chlorosis and necrosis of foliage, wilting often evident. Obtained at Coeur d'Alene Nursery.

Because of the extensive disease problem at Coeur d'Alene, we did not believe control seedlings should be obtained there. Instead, controls

2/ 4600 memorandum from Dr. Riffle to Oscar Dooling, Region 1, November 21, 1969.

were selected from the State of Montana nursery in Missoula, Montana, where no observable root disease problem was evident. Also, no effort was made to identify seed source of any of the species-disease combinations used in this study.

The quantity of seedlings outplanted in each species-disease intensity class was:[3/]

1. Douglas-fir, control - 100
2. Douglas-fir healthy - 100
3. Douglas-fir diseased - 200
4. Western larch, control - 100
5. Western larch, healthy - 100
6. Western larch, diseased - 200
7. Engelmann spruce, control - 100
8. Engelmann spruce, healthy - 200
9. Engelmann spruce, diseased - 100

An area 4 miles west of St. Regis, Montana, was chosen for the outplanting. The site was clearcut and broadcast-burned in 1968. Seedlings were planted on nearly flat terrain of a north exposure and *Abies grandis-Pachistima myrsinites* habitat type (Daubenmire and Daubenmire 1968). We assumed this habitat type would not be limiting to the growth or survival of any of the species used in this study (On 1971).

Seedlings were planted in May 1970. Each was referenced with a small red stake on which appropriate information was written. Total height to the nearest 0.5 inch was recorded for each at that time.

Final data for this report were taken in October 1972. (Three growing seasons elapsed between study establishment and final evaluation.) Seedlings were visually rated as:

1. Dead.
2. Diseased - chlorotic, some necrosis, short needles, etc.
3. Healthy - normal green, needles normal length.

Also each living seedling was measured for total height to the nearest 0.5 inch.

Determination of causal agents.--At the time of study establishment, 10 seedlings of each of the species-disease types (20 in Douglas-fir diseased, western larch diseased, and Engelmann spruce healthy), but

3/ Originally, the study was designed as a randomized complete block with two replications. However, because of problems in selecting nursery stock, we decided to report the study in this fashion--thus, the unequal quantities of seedlings in some categories.

different individuals than those used for outplanting were selected, cultured, and examined for possible pathogens. Two 1/2-inch stem segments, one at the root collar and the other about midstem, were placed aseptically on commercial potato-dextrose agar and allowed to incubate.

During a preliminary evaluation in June 1971, a small sample of seedlings that had died since planting were brought to the laboratory and cultured for possible pathogens as described above. No specific sampling scheme was followed.

RESULTS AND DISCUSSION

Outplanting.--All data reported here were taken in October 1972, three growing seasons following study establishment. Percent of seedlings healthy, diseased, or dead; average growth of healthy and diseased seedlings; and range of growth were computed for each species-disease combination. The data are summarized in table 1.

Table 1.--Summary of data collected in October 1972

Species	Disease intensity at time of planting	Condition of seedlings at evaluation			Average growth, inches		Range of growth, inches	
		Healthy	Diseased	Dead	Healthy	Diseased	Low	High
		- - - Percent - - -						
Douglas-fir	Control	58	4	38	4.0	1.0	0	6.0
	Healthy	60	11	29	5.0	.5	0	8.0
	Diseased	52	11	37	4.5	.5	0	9.0
Western larch	Control	65	5	30	9.0	3.0	0	22.5
	Healthy	65	5	30	11.0	4.0	0	22.5
	Diseased	48	2	50	11.0	3.5	0	17.5
Engelmann spruce	Control	66	4	30	5.5	2.0	0	12.0
	Healthy	74	4	22	6.5	2.0	0	12.5
	Diseased	63	6	31	5.5	2.5	0	12.5

In terms of percent healthy seedlings in 1972, there was little difference between the original control and healthy categories of Douglas-fir and western larch. However, for Engelmann spruce control was notably less than healthy, and only slightly greater than diseased. Based on this data, the best that could be expected in terms of healthy remaining seedlings would be 60 percent for Douglas-fir, 65 percent for western larch, and 74 percent for Engelmann spruce.

The percent healthy seedlings in 1972 was less for seedlings originally grouped as diseased than for healthy in all species. The data indicate

the diseased condition was responsible for an 8 percent reduction of remaining healthy Douglas-fir seedlings, 17 percent reduction in western larch, and 11 percent reduction in Engelmann spruce.

For all species the mortality was greater for seedlings originally classified as diseased than for those classified as healthy. The difference was most pronounced in western larch where 50 percent of the diseased seedlings died compared to 30 percent of the healthy.

Growth of seedlings classified as diseased in 1972 in all species-disease combinations was poor in comparison to those called healthy. The difference was a factor of about five in Douglas-fir and about three in western larch and Engelmann spruce. In contrast, diseased seedlings at time of outplanting but healthy in 1972 apparently overcame their diseased condition and assumed normal growth. Average growth of seedlings called healthy in 1972 was about the same, within species, for all the original disease intensity classes.

Our data support the thesis that western larch is a relatively fast-growing species at young age. Larch had the best average growth at 11 inches, followed by spruce at 6.5 inches, and Douglas-fir at 5. Even the larch seedlings classified as diseased in 1972 had better growth than either the diseased Douglas-fir or spruce. The best growth for any seedling was 22.5 inches by western larch. This is far more than the 12.5 inches by spruce or the 9.0 inches by Douglas-fir.

Causal agents.--A list of fungi isolated[4/] in 1970 and their relative abundance is given in table 2. *Fusarium oxysporum*, *Fusarium roseum*, *Phoma* sp., *Alternaria alternata* (Fries) Keissler, and *Trichoderma* spp. were the most abundant. The *Fusarium* spp. and *Cylindrocarpon* sp. nearly always appeared to grow from the cut end of the stem and root segments onto the agar. The other fungi appeared to grow from the surface of the stem cylinder.

Eighty-eight dead seedlings were collected during the preliminary evaluation in June 1971. Twenty-one were Douglas-fir, 36 were western larch, and 31 were Engelmann spruce. *Fusarium* spp. were isolated from all. Other microflora, including fungi, were not recorded.

Fusarium spp. and *Cylindrocarpon* spp. have been shown to be pathogenic on coniferous nursery seedlings. Tint (1945) showed *F. oxysporum* and *F. roseum* caused a 79 and 39 percent loss, respectively, of *Pinus resinosa* Ait. seedlings under laboratory controlled conditions. Much of the damage occurred as postemergence damping-off, but some was classified as root rot (later than damping-off). Hodges (1962, 1969) identified *Fusarium* spp. and *Cylindrocarpon* spp. as primary causes of

4/ Fungal identifications were confirmed by Charles E. Hodges, Southeastern Forest Experiment Station, Research Triangle Park, N. C.

Table 2.--Frequency of fungi isolated from coniferous seedlings at time of study establishment (April 1970)

Fungal species	Douglas-fir						Engelmann spruce						Western larch					
	Control		Healthy		Diseased		Control		Healthy		Diseased		Control		Healthy		Diseased	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Fusarium oxysporum	8	6	4	2	8	3	5	4	11	5	2	1	3	3	2	5	6	9
Fusarium roseum					2	1			3	1	2						5	
Phoma sp.	2	6	6	10	6	20	2		6	18	6	8	2	8	8	10	10	19
Ulocladium alternariae (Cke.) Simmons	1	1		2	2	2	1	1					1	1				
Alternaria alternata	1	6	2	2		3	2	3	1	4			2	5	1	1		9
Trichoderma spp.	4		2		1		8		10	1	7	2	4		3		1	
Cylindrocarpon sp.					2		1										1	
Penicillium spp.			1		3		1		2		1	1	3	2		1	1	1
Gleosporium sp.			1															
Gliomastix sp.									1									
Phialophora sp.									1						2			
Chaetomium globosum Kunze ex Fr.									1						1			
Verticillium sp.											4							
Thermomyces sp.														1				
Botryotrichum sp.													1					
Stemphylium sp.														2				
Total plates	10	10	10	10	20	20	10	10	20	20	10	10	10	10	10	10	20	20
Percent *Fusarium* spp.	80	60	40	20	80	30	50	40	75	30	40	10	30	30	20	50	60	90

root disease in southern pine nursery seedlings. Dr. Charles Hodges[5/], after examining many of the cultures obtained in this study, stated "about the only possible pathogens you have are the *Fusarium* spp. and *Cylindrocarpon*."

Based on the frequency with which *Fusarium* spp. were isolated from seedlings at time of study establishment and at the preliminary evaluation and because pathogenicity has been documented in the literature, it is highly probable that *Fusarium* spp. are the causal agents of the disease described in this study.

Our data show that *Fusarium* spp. were isolated from all classes and species of seedlings used in the study. As low as 10 percent incidence was found in diseased spruce while up to 80 percent was found in control and in diseased Douglas-fir and 90 percent in diseased larch. The fungus was found in both shoot and root tissue (table 2). The presence of this pathogen in control and healthy seedlings as well as in diseased at first appears inconsistent. However, Bloomberg (1966) isolated *Fusarium* spp. from 69 percent of the shoots and 50 percent of the roots of healthy Douglas-fir seedlings from a Canadian forest tree nursery. He found up to 66 percent *Fusarium* in diseased seedlings.

In a later study (Bloomberg 1971), he compared the relative pathogenicity of five different isolates of *Fusarium oxysporum* and concluded that different strains of the fungus varied in virulence. Strains of *F. oxysporum* causing postemergence damping-off likely do not cause root rot, and strains in healthy seedlings not affected by damping-off may become active later on to cause root rot. He surmised that change within the host probably stimulates the root rotting strains to cause disease.

CONCLUSIONS

This study was not designed to provide positive proof that one or more of the fungi isolated were the cause of conifer seedling disease and were definitely the cause of poor survival of outplanted stock. Rather, the intent was to show association between known pathogenic fungi, seedling disease, and outplanting survival. This was done. *Fusarium* spp. were isolated at high frequency from diseased nursery stock and from seedlings that died within 1 year of outplanting. Based on our data and on the available literuature, it is highly probable the *Fusarium* spp. are the causal agents. Inoculation tests would have to be done to prove this.

The percentage of diseased stock that recovered was very poor, even though seedlings were planted on an excellent growing site. Because of

5/ Forest Service memorandum dated July 6, 1970, from Dr. Hodges to Clinton E. Carlson.

the good productivity of the *Abies grandis-Pachistima myrsinites* habitat type, it is probable that as poor or poorer recovery would be expected on other habitat types. Up to 65-75 percent of the type of seedlings classified as "diseased" in this study are routinely shipped to the field for reforesting cutover areas. Because of the poor recovery of these seedlings, there is little question that outplanting them is a biologically unsound practice.

As mentioned before, Bloomberg (1971) speculated that a change within the host may be responsible for the ability of *Fusarium* spp. to cause disease after a seedling has hardened off. Any change within the host would be either directly or indirectly related to its external environment. It is possible that changes in the soil environment at the Coeur d'Alene Nursery during the last 5 or 6 years could be responsible for the disease condition that now exists. Programed soil fumigations eliminating natural enemies of pathogenic fungi, but not the latter, decreased organic matter (1 to 3 percent as recent nursery soil analysis reports indicate), increasing soil pH (7.5 in some sections), and high nitrogen could all be factors. The problem needs detailed study.

Survival of control and healthy seedlings, all species, was less than we expected. Seedlings were planted with extreme care and habitat type should have favored at least 80 percent survival. It is possible that the *Fusarium* spp. found in control and healthy seedlings contributed to the poor survival. Also, seed source may have been an important factor.

In conclusion:

1. *Fusarium* spp. are ubiquitous in seedlings at Coeur d'Alene Nursery, existing in healthy and diseased stock. They are also present in healthy seedlings at the State of Montana Nursery in Missoula, Montana.

2. *Fusarium* spp. likely are causing the diseased condition of much coniferous nursery stock at Coeur d'Alene. The disease probably becomes manifest due to a change within the host, and that change may be triggered by poor soil conditions.

3. Seedlings classified as diseased had a notably lower recovery than healthy.

4. Diseased seedlings surviving outplanting apparently overcame their diseased condition and assumed normal rates of height growth.

5. Engelmann spruce survived outplanting best, followed by Douglas-fir and western larch.

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